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New record and distribution extension of the endangered freshwater fish *Cnesterodon hypselurus* (Cyprinodontiformes: Poeciliidae) in the upper Paraná River basin, Brazil

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Abstract: *Cnesterodon hypselurus* is an endangered species from the Upper Paraná River basin, with distribution restricted to three streams with diminished habitat quality. An updated distribution including new locations in the Tibagi River basin is presented, increasing its area of occurrence and providing a new low-elevation record of 728 m. The recognition of these populations suggests that *C. hypselurus* might have a larger distribution in the east-central Paraná State region, changing to vulnerable the previously endangered status of the species.

Key words: endemism; habitat degradation; Tibagi River; Itararé River

Cnesterodon Garman, 1895 is a monophyletic genus of the family Poeciliidae, of the live-bearing Cyprinodontiformes. An extensive revision of the genus was made by Lucinda (2005) and Lucinda and Reis (2005), recognizing eight valid species, with two more recently described: *Cnesterodon holopteros* Lucinda, Litz & Recuero, 2006 and *Cnesterodon pirai* Aguilera, Mirande & Azpelicueta, 2009. These 10 species inhabit coastal and inland drainages in Argentina, southern Brazil, Paraguay and Uruguay, restricted to headwaters and high elevation regions of these basins (Lucinda 2003, 2005; Lucinda et al. 2006).

Cnesterodon hypselurus Lucinda & Garavello, 2001:129 is endemic to streams in Paraná State, Brazil, and, according to the literature (Lucinda and Garavello 2001; Shibatta et al. 2002; Lucinda 2003, 2005; Lucinda and Reis 2005; Langeani et al. 2007; Meredith et al. 2010; Pollux et al. 2014), its distribution is restricted to the Cilada and Lambari Rivers, both tributaries of the Capivari River, part of the Itararé River basin, and a nameless tributary of the Guaricanga River, of the Tibagi River basin. Both the Tibagi and Itararé River basins are part of the the Paranapanema drainage, flowing from South to North, approximately 550 km and 300 km in length respectively. Shibatta et al. (2002) proposed that *C. hypselurus* was endangered in the Tibagi River basin, however only recently the species was included in the Brazilian list of endangered species (MMA 2014). According to the IUCN Red List Categories and Criteria, it was classified as EN (B1ab(iii)), indicating that *C. hypselurus* is facing a very high risk of extinction, with a range of less than 5,000 km^2, and known from no more than five locations, with continuing decline of habitat quality (IUCN 2001; ICMBIO 2014). Herein we report new locations for *C. hypselurus* in the Tibagi River basin, increasing the known localities for this species and changing its threat category according to the IUCN parameters (IUCN 2001).

Field studies were conducted between February and November 2014, in first and second order stretches of eight streams in the middle Tibagi River basin, property of Klabin S.A. cellulose and paper company, east-central Paraná State. This area has been classified as possessing extremely high biological importance and high priority for conservation (MMA 2007). A total of 38 specimens of *Cnesterodon hypselurus* (Figures 1 and 2) were sampled in one of the eight streams, in a first order stretch of the Arroio do Gica stream (24°12′32.78″ S, 050°36′35.65″ W, 728 m), tributary of the Tibagi River, Telêmaco Borba (Figure 3).

Figure 1. Specimens of *Cnesterodon hypselurus* showing coloration in life in male (above) and female (below). Specimens not preserved. Photos by F.C. Jerep.

Figures 2. Coloration of alcohol preserved specimens of *Cnesterodon hypselurus*. Mature male, MZUEL 12699, 24.1 mm SL (above) and female, MZUEL 12640, 34.5 mm SL (below). Photos by J.F.M. Silva.

Specimens were collected in pool habitats (according to Rincón 1999) using a seine net (2 mm mesh) and sieves (2 mm mesh) for one hour, under SISBIO permit #42829-1. Fishes were anesthetized with clove oil (Anderson et al. 1997; Lucena et al. 2013), fixed in 10% formalin for 48 hours, washed in running water and preserved in 70% ethanol (Uieda and Castro 1999). Voucher specimens analyzed in this study are deposited

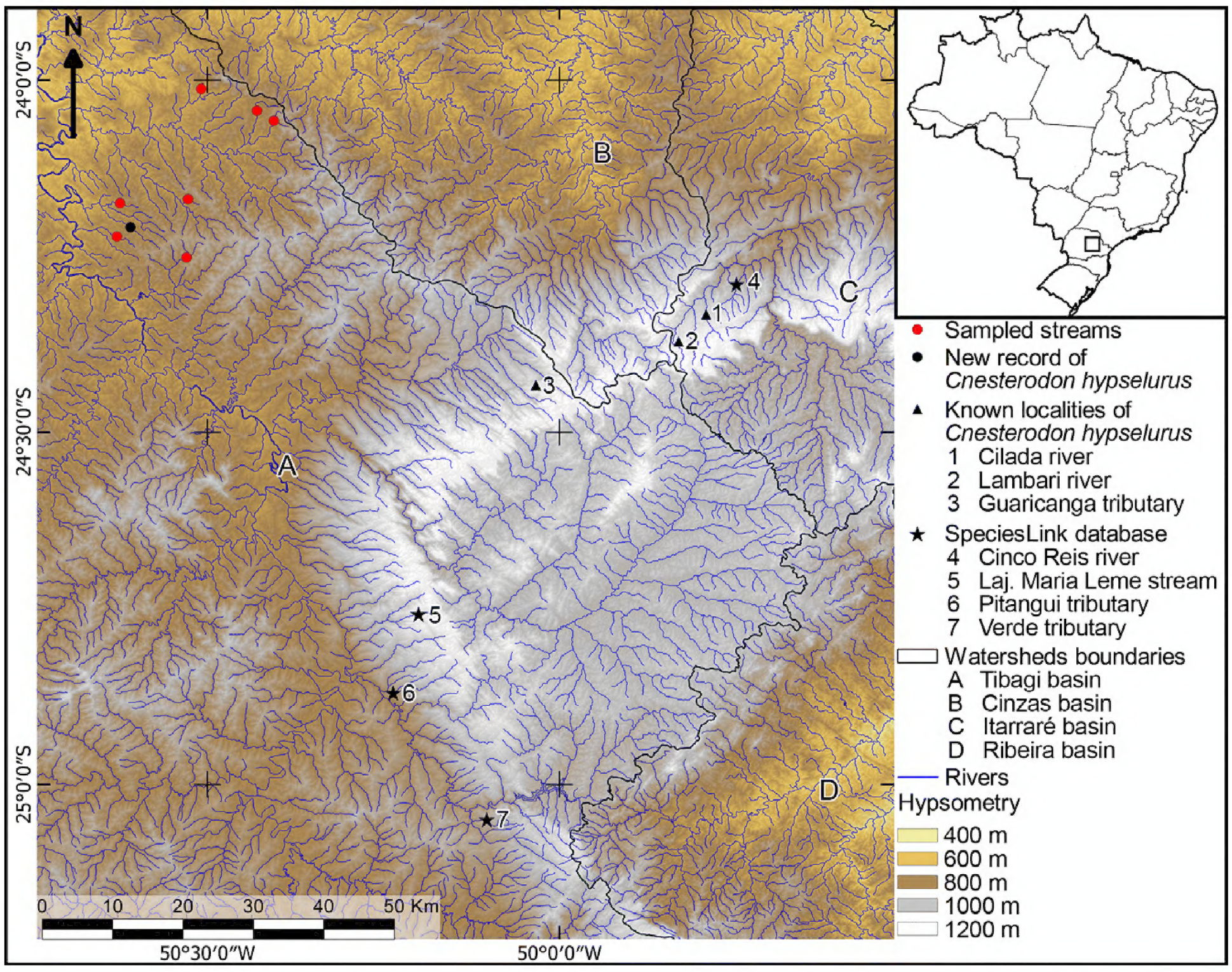

Figure 3. Updated geographic distribution of *Cnesterodon hypselurus*. Additional sites in red represent seven streams sampled during the present study. Source: adapted from Miranda (2005), SUDERHSA (2007) and IBGE (2013).

at the Museu de Zoologia da Universidade Estadual de Londrina (MZUEL) under the following catalogue numbers: MZUEL 12640, 12699, 12752 and 12798.

Two other species were collected in the same stream: *Astyanax paranae* Eigenmann, 1914 and *Trichomycterus davisi* (Haseman, 1911). *Astyanax paranae* inhabits the same pool habitat where *C. hypselurus* was collected, while *T. davisi* was collected strictly in riffle habitat.

The specimens were identified as *Cnesterodon hypselurus* following the identification key and diagnosis proposed by Lucinda (2005) and comparisons with the paratypes (MZUEL 1600), specimens of congeners (Lucinda et al. 2006; Aguilera et al. 2009), and specimens identified by P.H.F. Lucinda (MZUEL 1727 and MZUEL 1728). The updated distribution map of the species (Figure 3; Table 1) was based on data from literature (Lucinda and Garavello 2001; Shibatta et al. 2002; Lucinda 2003, 2005; Lucinda and Reis 2005; Langeani et al. 2007; Meredith et al. 2010; Pollux et al. 2014) and from the SpeciesLink database (CRIA 2015). Vouchers from new localities found in SpeciesLink were examined to verify identification.

Sites where specimens were collected were included in a GIS base map containing limits of the Paraná State Watersheds (SUDERHSA 2007), Digital Elevation Model (Miranda 2005) and hydrography (IBGE 2013). The new extent of occurrence was calculated by the area of a minimum convex polygon, which is the smallest polygon with no internal angle exceeding 180° and containing all sites of occurrence (IUCN 2001).

According to Lucinda (2005), *Cnesterodon hypselurus* can be identified by two autapomorphies, the presence of a dark longitudinal band along the flank in preserved material (Figure 2), and the bony style of ray 3 of the gonopodium of fully developed males curly-bracket shaped, with a constriction located at two-thirds of its length (Figure 4). In addition, the combination of the dark brown blotches along body sides forming bars and covering more than four scales in a transverse row, the presence of a dark band along flank and depth of the caudal peduncle in adult males (15.5–17.8% SL) differentiate this species from its congeners. A variation in the number of segments at the tip of ray 4a of the gonopodium of mature males was observed, with four to six segments before the claw-shaped bony tip. The specimens also presented some variation in the coloration pattern (Figure 2) in comparison to the original description and specimens from other localities. The longitudinal dark stripe may be slightly fainter in some specimens, and the dark vertical bars might cover up to three scales of a transverse row instead of four, as mentioned in the original description (Lucinda and Garavello 2001). Notwithstanding, the variation herein observed is similar to the color pattern of the examined type material and photographs (Lucinda and Garavello 2001, fig. 5; Lucinda 2005, fig. 7).

The pool habitat where the specimens were collected at Arroio do Gica stream is 3.5–4.0 m wide, 40–59 cm deep, and 0.04–0.06 m/s current speed. The bottom of the stream was composed of rocks, leaf litter and non-consolidated clay (Figure 5). Riparian forest covered the right margin while the left margin was bordered by commercial cultivation of *Pinus* sp. that was harvested during the studies. The water temperature ranged from 15.4 to 19.8°C, pH 6.3 to 7.7, conductivity 15.2 to 25.1 µS/cm and dissolved oxygen 7.6 to 8.8 mg/L (80.5–88.1%).

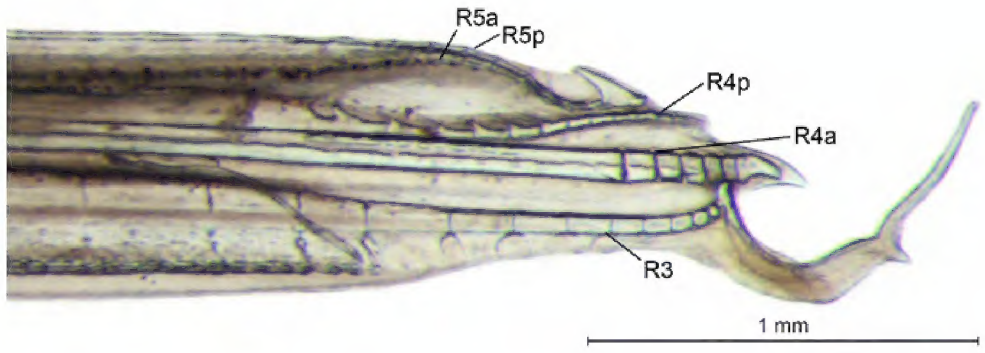

Figure 4. Distal tip of the gonopodium of a mature male of *Cnesterodon hypselurus*, MZUEL 12699, 25.2 mm SL. R3, R4a, R4p, R5a, R5p are sections of the anal-fin rays, modified in gonopodial rays. Left side, lateral view. Photo by F.C. Jerep.

Table 1. Known localities of *Cnesterodon hypselurus* in Paraná State and vouchers. Elevation in meters above sea level; *N* = number of specimens.

Locality	Drainage	Municipality	Geographic coordinates	Altitude	Catalog number (*N*)
Cilada River[7, 8]	Itararé	Jaguariaíva	24°19′58.03″ S, 049°47′30.22″ W	1,078 m	MCP12593 (31)[1, 4, 5], MCP22743 (1)[1, 3, 4, 6], MCP22744 (9)[1, 4, 5], MHNCI5890 (10)[1, 4], UMMZ215219 (20)[1, 4], USNM356395 (2)[1, 4], MZUEL1728 (122)[1, 4], MZUSP85935.0 (100)[9], UFRGS18402 (32)[9]
Lambari River	Itararé	Jaguariaíva	24°22′24.28″ S, 049°50′23.47″ W	1,136 m	MCP12602 (7)[1, 4], MCP22745 (5)[1, 4], MHNCI5892 (3)[1, 4]
Cinco Reis River	Itararé	Jaguariaíva	24°17′27.70″ S, 049°44′54.30″ W	941 m	MZUSP85936.0 (3)[9]
Guaricanga River tributary[2]	Tibagi	Piraí do Sul	24°25′60.00″ S, 050°01′60.00″ W	1,110 m	MZUEL1600 (14)[1, 4], MZUEL1727 (10)[1, 4], MCP23326 (10)[4]
Arroio do Gica stream	Tibagi	Telêmaco Borba	24°12′32.78″ S, 050°36′35.65″ W	728 m	MZUEL12640 (14), MZUEL12699 (14), MZUEL12752 (6), MZUEL12798 (4)
Lajeado Maria Leme stream	Tibagi	Castro	24°45′34.00″ S, 050°11′60.00″ W	1,102 m	NUP15659 (10)[9]
Pitangui River tributary	Tibagi	Carambeí	24°52′16.00″ S, 050°14′10.00″ W	861 m	NUP15828 (6)[9]
Verde River tributary	Tibagi	Ponta Grossa	25°03′04.00″ S, 050°06′11.02″ W	904 m	DZSJRP20376 (168)[9]

[1]Lucinda and Garavello 2001; [2]Shibatta et al. 2002; [3]Lucinda 2003; [4]Lucinda 2005; [5]Lucinda and Reis 2005; [6]Langeani et al. 2007; [7]Meredith et al. 2010; [8]Pollux et al. 2014; [9]CRIA 2015.

Figure 5. Photograph of the new record locality of *Cnesterodon hypselurus* in Arroio do Gica stream, Telêmaco Borba, PR, Brazil. Photo by S.T. Benemann.

The stream habitat where the species has been previously recorded were not described in the literature, although *C. hypselurus* lives in clear waters with little flow in the Tibagi basin (Shibatta et al. 2002). The other streams sampled during the study did not have pools with similar characteristics as found in the Arroio do Gica, indicating a possible habitat requirement of this species. The elevation of the previous known locations ranged from 861 to 1,136 m above sea level, but Arroio do Gica stream is at 728 m above sea level, making it the lowest site reported for the species.

New records from the SpeciesLink database indicated four additional locations for the species (CRIA 2015), for a total of eight different streams (Figure 3, Table 1). The updated extent of occurrence for *C. hypselurus* was found to be 3,989 km^2. Although the updated area of distribution still fits the criteria for EN(B1) (extent of occurrence less than 5,000 km^2), the occurrence in eight different locations invalidates the criteria EN(B1a) (species known to exist at no more than five locations). Based on the abovementioned, the category of the species changes from endangered (EN) to vulnerable (VU) according to the VU(B1ab(iii)) criteria: extent of occurrence restrict to less than 20,000 km^2 and known to exist at no more than ten locations with continuing decline of habitat quality.

The vulnerable category still indicates that the species is facing a high risk of extinction. Additional data about reduction in population size, number of mature individuals in the population and probability of extinction in the wild in five generations are still needed for the species, and can only be achieved through standardized sampling in the locations for an extended period of time.

The present study increases the known distribution of *Cnesterodon hypselurus* to eight localities; changing the conservation status of the species according to the IUCN (2001) parameters to VU(B1ab(iii)). The population recorded herein is relatively small and should be regularly monitored. On the other hand, the discovery of *C. hypselurus* in this stream suggests that there may be additional undiscovered populations in the Tibagi, Cinzas and Itararé River basins. We encourage targeted inventories efforts in streams with similar habitat characteristics as observed in Arroio do Gica stream throughout this region, as well as a phylogeographic study to determine the processes involved in the geographic distribution of this species.

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